Hunting the RNA "Slicer"

NSLS users may have found a key player in RNA interference

As the result of work done at the NSLS, scientists from Cold Spring Harbor Laboratory have very likely determined the identity of a sought-after protein that is vital to RNA interference (RNAi). RNAi is fundamental cellular process intimately involved in the development and virus-fighting ability of all organisms, as well as gene expression — how genes produce certain cell features. The researchers' result, the crystal structure of the protein, will significantly impact the field of biology by helping to illuminate the details of these mechanisms.

Before the protein was identified, biologists only knew that there should be a protein, dubbed the "Slicer," that performed a critical role in RNAi. It received this nickname for the function the scientists suspected it carried out: slicing, or cleaving, strands of messenger RNA into pieces, much like a pair of molecular "scissors." Messenger RNA is the type of RNA that decodes the information contained in DNA (i.e. genes) and carries it out of the cell nucleus, where it is used to synthesize proteins. The Slicer is one component of a large multi-protein structure, called the RNA-induced silencing complex (RISC), that "interferes" with messenger RNA's mission.

This research team is the first to discover very convincing evidence that a certain protein is, in fact, the Slicer. The protein is known as Argonaute. It is discussed in the group's research paper on the work, which appears in the September 4, 2004 edition of *Science*.

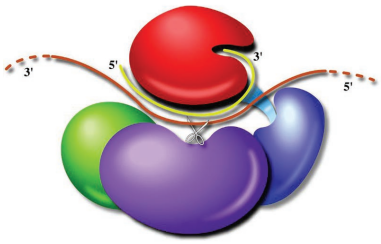
Authors (from left) Leemor Joshua-Tor, Gregory J. Hannon, Ji-Joon Song, and Stephanie K. Smith

"The crystal structure of Argonaute contained a clue that led us to identify it as the Slicer," said Leemor Joshua-Tor, a structural biologist and the study's lead researcher. "We observed that an important structural feature on Argonaute was very similar to that of another enzyme already known to cleave RNA."

The group found that Argonaute is composed of a large crescent-shaped base and a smaller globular region that sits over it, tethered by a thin stalk-like region. The crescent has its own sub-structure, made up of three distinct parts, or "domains" — a center domain and two outer ones.

Based on these features, Joshua-Tor and her colleagues postulated how Argonaute might act as the Slicer. In their scenario, a strand of "small interfering" RNA (siRNA), which is a short type of RNA created earlier in the interference process, binds to Argonaute and guides it to a complementary strand of messenger RNA. The siRNA positions the messenger RNA in the groove created by Argonaute's crescent and globular segments. Once in place, the crescent's "PIWI" domain cleaves the messenger RNA, leaving the siRNA intact.

"This is an important result, but many questions still remain," said Joshua-Tor. "For example, we still do not know how Argonaute proteins participate in developmental processes."



A schematic depiction of the model for siRNA-guided messenger RNA cleavage. The siRNA (yellow) binds with its 3' end in the cleft in the globular domain (red). The 5' end is predicted to bind near the other end of the cleft. The messenger RNA strand (brown) comes in between the crescent's N-terminal (blue) and globular domains and out between the globular domain and the crescent's middle domain (purple).

Gene silencing during RNAi (the act of blocking gene expression) may sound destructive, but the process can prevent messenger RNA from carrying out the orders of potentially malicious genes — genes for defects, for example. RNAi also appears to play an important role in normal organ function. Currently, biologists are experimenting with ways to silence specific genes for medical purposes.

Joshua-Tor and her colleagues, including student researcher Ji-Joon Song, collected data at NSLS beamline X25 and later used the data to determine Argonaute's structure. Using protein crystallography, they directed a beam of x-rays at a crystal of Argonaute protein and used a detector to collect the x-rays as they scattered away from the atoms in the crystal. The researchers then analyzed this pattern, which is unique to Argonaute, to create a three-dimensional model of the protein.

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*For more information, see: J.J. Song, S.K. Smith, G.J. Hannon, and L. Joshua-Tor, "Crystal Structure of Argonaute and its Implications for RISC Slicer Activity," Science, **305**, 1434-1437 (2004).*

— Laura Mgrdichian